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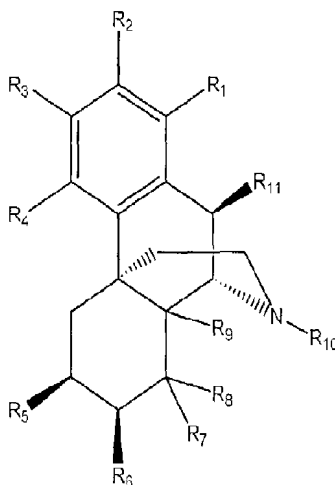
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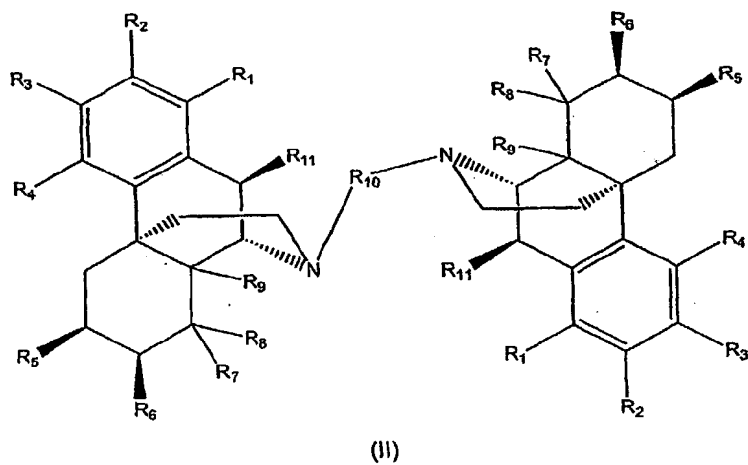
(54) **Alkaloid compounds and their use as anti-malarial drugs**

(57) The present invention concerns the use of compound of formula I



or

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and their pharmaceutically acceptable salts in the preparation of a pharmaceutical composition useful namely in an anti-malarial prophylactic or curative treatment. The invention also concerns the new compounds and the pharmaceutical compositions comprising the same.

## Description

[0001] The present invention concerns alkaloid compounds and their use as a medicament. These compounds and derivatives are particularly useful against malaria in a prophylactic and/or curative treatment. Therefore, the present invention also concerns pharmaceutical compositions comprising the new compounds and the use of the compounds in a process for the preparation of anti-malarial compositions. Furthermore in another aspect of the invention, it concerns a process of preparation of these compounds.

[0002] Malaria is a serious health care problem posing a great menace to society due to the number of patients infected and the mortality rate of patients, as evidenced by about 300 million patients attacked annually predominantly in tropical and subtropical regions, causing about 2 million deaths in these areas.

[0003] Malaria is usually treated by administering chloroquine, pyrimethamine, quinine, proguanil, primaquine, artemisinin compounds, etc..., even combinations thereof, but effective treatments have become difficult with these conventional anti-malarial drugs because most of the malarial parasites eventually become resistant to these anti-malarial drugs.

[0004] Some of the known chemical compounds to treat malaria are not free of side effects, this rendering their long-term use deleterious in some aspects and limiting the use of these compounds.

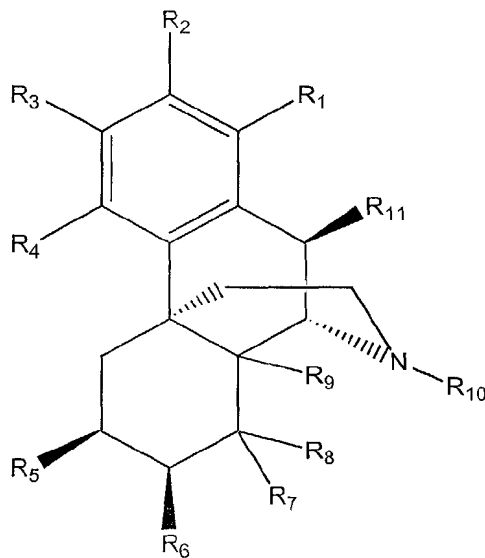
[0005] There is therefore still a need for compounds having an efficiency against malaria, without their usual drawbacks. Furthermore, there is a need for anti-malarial drugs which are easy to formulate in pharmaceutical compositions.

[0006] The applicants have now found that some compounds isolated from *Strychnopsis thouarsii* and also synthetic derivatives thereof have good anti-malarial activity, particularly on the hepatic stage (also call exoerythrocytic stage) of *Plasmodium*, contrary to most of conventional antimalarial drugs which are only active on the erythrocytic cycle.

[0007] JP 62-263158 describes sinococuline as an anti-tumoral active agent. JP 62-289 565 describes tetracyclic alkaloids and their anti-tumoral activity.

[0008] The present invention thus concerns the use of the compounds of formula I or II below and their derivatives as medicaments, particularly as anti-malarial compounds.

[0009] Amongst them, most are new. In another aspect, the invention concerns chemical compounds having the formula



(I)

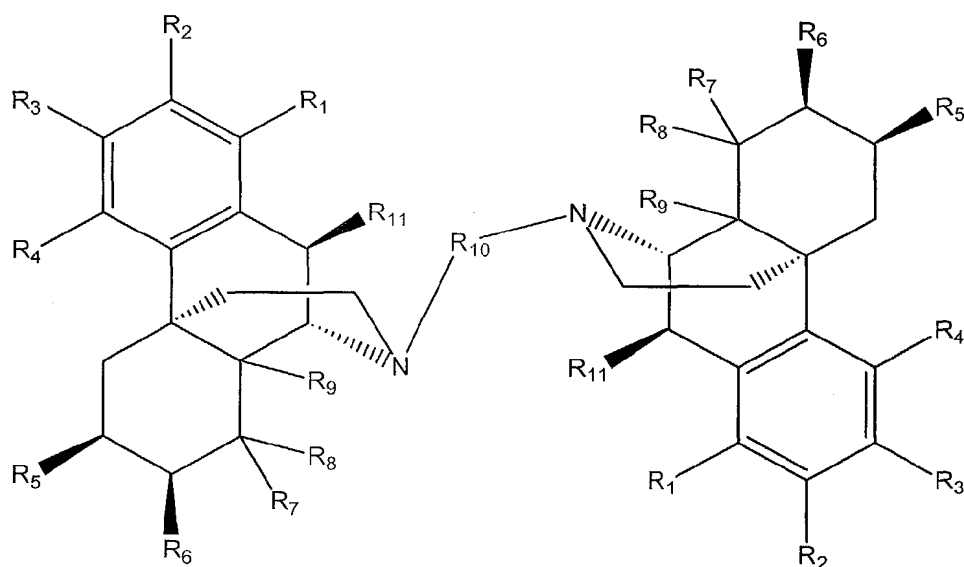
wherein

- $R_1$  is H or  $OC_iH_{2i+1}$  with  $i$  between 0 and 6;

- $R_2$  is H or  $OC_jH_{2j+1}$  with j between 0 and 6;
- $R_3$  is H or  $OC_kH_{2k+1}$  with k between 0 and 6;
- 5 -  $R_4$  is H or OH;
- $R_5$  is OH or  $OC_mH_{2m+1}$  with m between 1 and 6 or an acetoxy group or an oxo group;
- $R_6$  is OH or  $OC_nH_{2n+1}$  with n between 1 and 6 or an acetoxy group or an oxo group;
- 10 -  $R_7$  is  $OC_pH_{2p+1}$  with p between 0 and 6;
- $R_8$  and  $R_9$  may be similar or different and represent H or  $OC_qH_{2q+1}$  with q between 0 and 6, or Hal where Hal is Cl, Br, F or I, or form together a covalent bond whereby the bond is a double bond or form with an oxygen atom an ether bond (epoxy group);
- 15 -  $R_{10}$  is H or  $C_rH_{2r+1}$  with r between 1 and 12 or an unsaturated alkyl group or a cycloalkyl group (tri- to hexa-) unsaturated or not, with or without heteroatoms; or an aromatic or a polycyclic aromatic group with or without heteroatoms; or  $C_sH_{2s}-A$  with s between 1 and 12 and whereby A is a saturated or (tri- to hexa-) unsaturated cycloalkyl group, with or without heteroatoms or an aromatic or a polycyclic aromatic group with or without heteroatoms or A is  $C_6H_{5-t}-(Hal)_t$  with t between 1 to 5 or  $C_6H_{5-u}-(O-C_vH_{2v+1})_u$  with u between 1 to 5 and v between 0 and 6; or  $R_{10}$  represents two substituents similar or different rendering the nitrogen atom quaternized, or an oxygen atom (nitron group) and in which case the bond between the nitrogen atom and  $C_9$  is a double bond; or  $R_{10}$  represents  $CH_2CH_2[OCH_2CH_2]_wOCH_2CH_2-B$  with w between 0 and 10 and where B is OH, O-D or NH-D where D is a  $C_1-C_{12}$  alkyl group bearing an electrophilic function such as an isothiocyanate;
- 20 -  $R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with x between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group;

or  $R_{10}$  and  $R_{11}$  may represent an isoalkylidene group;

or



(II)

wherein  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  are defined as above,

- $R_4$  is H or OH or  $OC_lH_{2l+1}$  with  $l$  between 2 and 6;
- $R_{10}$  is  $C_yH_{2y}$  with  $y$  between 1 and 12 or  $CH_2CH_2[OCH_2CH_2]_zOCH_2CH_2$  with  $z$  between 0 and 10; and

$R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with  $x$  between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group;

and all stereoisomers and optical isomers thereof,

with the proviso that in formula (I) (1)  $R_2$  is not H or alkoxy when  $R_3$  is OH or alkoxy and  $R_4$  is H or OH and  $R_5$  and  $R_6$  are OH or alkoxy and  $R_7$  is  $OCH_3$  and  $R_8$  and  $R_9$  represent a double bond and  $R_{11}$  is H; and (2)  $R_1$  is not H when  $R_2$  is H and  $R_3$  and  $R_7$  are  $OCH_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are OH and  $R_8$  and  $R_9$  represent a double bond and  $R_{10}$  is H, which are known from JP 62-263158 and JP 62-289565.

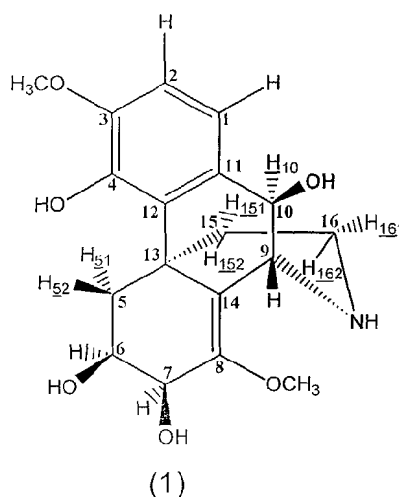
**[0010]** Particularly, the invention concerns the compounds of formula I with the above proviso and the use of compounds of formula I without the proviso for their antimalarial activity. Amongst them, the preferred compounds are those wherein  $R_8$  and  $R_9$  form a double bond, and  $R_1$  and  $R_2$  are H, and particularly the compounds wherein  $R_{11}$  is OH.

**[0011]** According to an aspect of the invention,  $R_{10}$  can represent H or  $C_rH_{2r}-A$  with  $r$  between 1 and 12 and whereby A is H or a cycle  $C_sH_{2s-1}$  with  $s$  between 3 and 6 or an aromatic cycle or an aromatic polycycle or an aromatic cycle substituted as  $C_6H_{5-t}(Hal)_t$  with  $t$  between 1 to 5 and where Hal is Cl, Br, F or I, or as  $C_6H_{5-u}-(O-C_vH_{2v+1})_u$  with  $u$  between 1 to 5 and  $v$  between 0 to 6, or as  $C_6H_{5-w}(NH_2)_w$  with  $w$  between 1 to 2; or  $R_{10}$  represents two substituents similar or different rendering the nitrogen atom quaternized, or an oxygen atom; or  $R_{10}$  represents  $CH_2-CH_2-[O-CH_2-CH_2]_xO-CH_2-CH_2-B$  with  $x$  between 0 and 10 and whereby B is H or OH or  $NH_2$  or  $N=C=S$ .

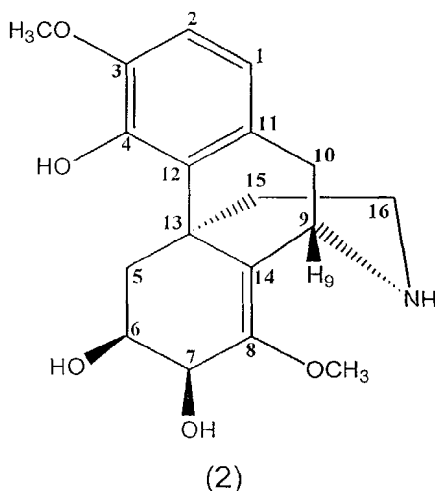
**[0012]** The preferred compounds are described hereafter namely in the examples and the detailed description.

**[0013]** More particularly, the invention thus concerns 4, 6, 7, 10-tetrahydroxy- 8, 14- didehydro- 3, 8- dimethoxymorphinan and its derivative *N*-methyl-, *N*-propyl-, *N*-4-methoxybenzyl-, *N*-4-hydroxybenzyl-, *N*-4-bromobenzyl- or *N*-cyclopentyl- - 4, 6, 7, 10- tetrahydroxy- 8, 14- didehydro-3, 8- dimethoxy-morphinan, as well as their pharmaceutically acceptable salts, and their optical isomers including racemates, and more particularly the isomers having the optical configuration (6S, 7S, 9R, 10R, 13S).

**[0014]** More particularly, the invention concerns



and the use of (2)



as anti-malarial compounds.

**[0015]** Furthermore, the invention concerns pharmaceutical compositions containing the compounds of formula I or II as above defined and stereoisomers and optical isomers and pharmaceutically acceptable salts thereof as an active agent together with an pharmaceutically acceptable vehicle, particularly an aqueous vehicle.

**[0016]** It also concerns pharmaceutical compositions comprising a compound of formula I or formula II as above defined, without the proviso together with at least one second anti-malarial compound and a pharmaceutically acceptable vehicle.

**[0017]** It also concerns the use of these compounds for the preparation of a pharmaceutical composition useful in the treatment of malaria, said composition comprises at least a compound of formula I or II without the proviso and another anti-malarial compound.

**[0018]** The invention further concerns the use of the compounds of formula I or II without the proviso and all the stereoisomers and optical isomers thereof, for the preparation of a pharmaceutical composition useful in the prophylactic or curative treatment of malaria.

**[0019]** The compounds of the invention have an anti-malaria activity particularly drawn to the hepatic stage, which corresponds to the passage of the parasite into the liver and to the infection of the hepatic cells in which the parasites develop to form the hepatic schizonts which, when they are mature (for example in the case of *P. falciparum* on the 6<sup>th</sup> day after penetration of the sporozoites) release hepatic merozoites by bursting. The third stage is characterized by the infection of the blood erythrocytes by the asexual forms (merozoites) of the parasite; this erythrocytic stage of development corresponds to the pathogenic phase of the disease. The fourth stage corresponds to the formation of the forms with sexual potential (or gametocytes) which will become extracellular sexual forms or gametes in the mosquito. The second stage is also called the exoerythrocytic stage of Plasmodium.

**[0020]** The only known and practically used molecules that are active against the hepatic stage are primaquine and atovaquone.

**[0021]** Their use is however limited due to the very toxic nature of primaquine, and due to the rapid development of resistance for atovaquone which is therefore only used in combination with other anti-malarial drugs.

**[0022]** The compounds of the invention are particularly useful for prophylactic and curative treatment of the hepatic stage of malaria.

**[0023]** Decoction and methanolic extract of the plant were evaluated for their in vitro antimalarial activity through inhibition of Plasmodium growth in murine and human hepatocytes, according to tests as described below.

**[0024]** A bioassay-guided fractionation of the plant extract, linking this in vitro test to chromatographic separation techniques was carried out and led to the isolation of active compounds, as described below.

**[0025]** The invention therefore also encompasses isolated extracts of bark or leaves of the plants described therein for use in a pharmaceutical compositions.

**[0026]** The pharmaceutical compositions of the present invention contain a pharmaceutically acceptable carrier in addition to the invention compound(s). The pharmaceutically acceptable carrier depends on the dosage form.

**[0027]** One of the advantages of the invention compounds is their bioavailability. Their solubility in water and aqueous vehicles is a major advantage.

**[0028]** Amongst the pharmaceutically acceptable vehicle, aqueous vehicle are preferred.

**[0029]** When the pharmaceutical compositions are used for oral administration, they may appropriately contain phar-

maceutically acceptable carriers including binders such as dicalcium phosphate; disintegrants such as sucrose; dyes; and perfumes such as orange flavor; and solvents such as water, ethanol and glycerol.

**[0030]** When pharmaceutical compositions of the present invention are injectable compositions, suitable pharmaceutically acceptable carriers include sterilized water, isotonic saline and pH buffers. Alternatively, injectable compositions of the present invention may be sterilized powder compositions or lyophilized powder compositions that can be used by simple dissolution in sterilized water. Injectable pharmaceutical compositions of the present invention may contain sugars (glucose, mannitol and dextran, etc.), polyhydric alcohols (glycerol, etc.), and inorganic salts (sodium salts and magnesium salts, etc.).

**[0031]** When pharmaceutical compositions of the present invention are administered by intravenous injection or infusion, they may contain nutrients such as glucose, vitamins, amino acids and lipids.

**[0032]** Pharmaceutical carriers to be added to dosage forms for other administration modes such as nasal administration, inhalation and transdermal administration are also well-known to those skilled in the art.

**[0033]** When pharmaceutical compositions of the present invention are orally administered, they may be in the form of controlled- or sustained-release formulations. Well-known sustained-release formulations include ordinary sustained- or controlled-release formulations such as gel-coated formulations and multicoated formulations as well as site-specific delivery formulations (e.g., burst release at pyloric regions or effervescent delivery to the duodenum). Oral compositions include, for example, tablets, pills, capsules, ampoules, sachet, elixir, suspensions, syrups, etc.

**[0034]** The dosage forms and pharmaceutical carriers mentioned above are described in Remington's Pharmaceutical Sciences, 16<sup>th</sup> ed. (1980), Mack Publishing Company, which is incorporated herein by reference.

**[0035]** Pharmaceutical compositions or unit dose systems of the present invention can be administered via various other routes such as transmucosal (sublingual, nasal, buccal), enteral, dermal administration, suppositories or intravenous infusion. These administration modes depend on the amount of the active compound to be administered, the condition of the patient and other factors.

**[0036]** Among these administration modes, oral administration is especially preferred as well as any mode where the use of aqueous vehicle is of interest.

**[0037]** According to the invention the inventive compound is present in an amount of between 0,001 and 50% by weight of the pharmaceutical composition.

**[0038]** When present in combination with a second anti-malarial compound or as an enhancer of the anti-malarial activity of another anti-malarial compound, the inventive compound is present in an amount between 0,0001 and 20% by weight of the pharmaceutical composition.

**[0039]** In the present invention, the effective amount of inventive compound used for the treatment of malaria is normally 1-1,000 mg/kg weight daily, preferably 5-500 mg/kg weight daily depending on the age, body weight and condition of the patient and the administration mode.

**[0040]** Apart from their obtention by the extraction process, the compounds of the invention can be obtained by synthesis. As it appears from the following examples, the derivatives of (1) and (2) can be prepared according to usual chemical procedures. Particularly alkylation procedures are carried out on products (1) and (2) under suitable alkylation conditions with the corresponding suitable alkylating agents. For example suitable alkylating agents are the corresponding aldehydes or dialdehydes. Suitable conditions can be reductive alkylation conditions.

**[0041]** Furthermore, oxidation and subsequent alkylation can be carried out by usual procedures, and protection of the substituents previously performed, if necessary.

**[0042]** In the following examples, stem bark has been treated to extract the compounds from the plant. However, other parts of the plant might be treated, as well as other species able to produce similar extracts. The leaves of *Strychnopsis thouarsii* can also be used, as well as other plants and species. The person skilled in the art can adapt the separation process to the species or part of the plant to be treated.

## Preparation Examples

### Example 1: Extraction process

**[0043]** 500 g powdered *Strychnopsis thouarsii* stem bark was soaked under stirring in MeOH at room temperature for six days, according to the following procedure.

**[0044]** Each 24 hours, the solute was filtrated and the solid residue was treated with 2 l MeOH. The resulting six solutes were pooled and evaporated to dryness under reduced pressure, providing 22 g of methanolic extract.

**[0045]** This extract was dissolved in 2 l H<sub>2</sub>O and centrifugated at 10,000 rpm for 1 Hour. A residue of 10 g was removed, while the supernatant was partitioned three times between H<sub>2</sub>O and CH<sub>2</sub>Cl<sub>2</sub> (500 mL).

**[0046]** The resulting aqueous extract of 11.7 g was chromatographed on a reversed phase silica gel column (RP-2), eluted with a discontinuous gradient of H<sub>2</sub>O-MeOH in the proportion of (100 - 0, 90 - 10, 80 - 20, 0 - 100).

**[0047]** The fractions were pooled according to their TLC profiles into 3 fractions F1 to F3.

[0048] The fraction F1 (9.4 g) was further separated on a silica gel column, eluted with  $\text{CH}_2\text{Cl}_2$ -MeOH- $\text{NH}_4\text{OH}$  (85-15-0.5) leading to 10 fractions F1-1 to F1-10.

[0049] The fraction F1-6 was determined to be the most active amongst the obtained fraction, by an evaluation of the biological activity carried out according to the method as described below.

#### Example 2: Isolation of compounds (1) and (2)

##### [0050]

Compound (1) = 4, 6, 7, 10- tetrahydroxy- 8, 14- didehydro- 3, 8-dimethoxy-morphinan  $\text{C}_{18}\text{H}_{23}\text{NO}_6$

Compound (2) = 4, 6, 7- trihydroxy- 8, 14- didehydro- 3, 8-dimethoxy-morphinan (Sinococuline or FK1000) [CAS Registry Number: 109351-36-2]  $\text{C}_{18}\text{H}_{23}\text{NO}_5$

[0051] An aliquot of fraction F1-6 (0.850 g) was chromatographed on a sephadex gel column (LH-20) eluted with MeOH, leading to 4 fractions, F1-6-1 to F1-6-4.

[0052] The fraction F1-6-3 (0.368 g) was purified on a silica gel column eluted with  $\text{CH}_2\text{Cl}_2$ -MeOH- $\text{NH}_4\text{OH}$  (87-13-1), leading to 6 fractions, F1-6-3-1 to F1-6-3-6.

[0053] The fraction F1-6-3-4 (0.122 g) was shown to be a mixture of the two compounds (1) and (2).

[0054] An aliquot of F1-6-3-4 (0.045 g) was further submitted to a preparative silica gel TLC migrating in  $\text{CHCl}_3$ -NH  $(\text{C}_2\text{H}_5)_2$  (70-30), leading to 2 fractions, F1-6-3-4-1 (0.01 g) and F1-6-3-4-2 (0.028 g).

[0055] The first fraction was submitted to a silica gel column filtration with  $\text{CH}_2\text{Cl}_2$ -MeOH- $\text{NH}_4\text{OH}$  (70-30-3), providing pure compound (2) (0.004 g), while the second fraction, under the same conditions, afforded pure compound (1) (0.019 g).

#### Example 3: Isolation of compounds (1) and (2)

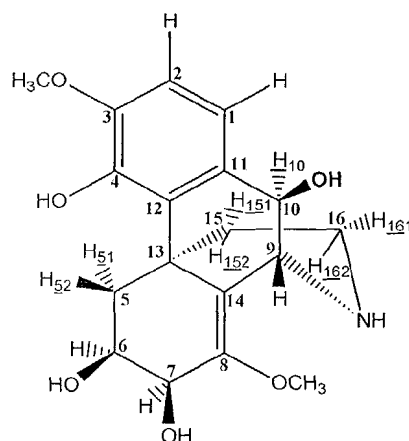
[0056] An aliquot of fraction F1-6 (0.22 g) was straightly subjected to a preparative silica gel TLC migrating in  $\text{CHCl}_3$ -MeOH- $\text{NH}_4\text{OH}$  (75-25-3.5), providing compound (1) (0.04 g), while bands corresponding to less polar products were pooled and submitted to a second preparative TLC migrating in  $\text{CHCl}_3$ -MeOH- $\text{CH}_3\text{COOH}$ - $\text{H}_2\text{O}$  (75-18-4-3), leading to 3 fractions, F1-6-1' to F1-6-3'.

[0057] The fraction F1-6-1' (0.012 g) was subjected to a silica gel column filtration with  $\text{CH}_2\text{Cl}_2$ -MeOH- $\text{NH}_4\text{OH}$  (90-10-1), providing pure compound (2) (0.004 g).

#### Example 4: Analytical data of compounds (1) and (2)

##### 1) Compound (1)

##### [0058]



(1)



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a) NMR data of (1) in CD<sub>3</sub>OD and its attribution by 2D NMR (400 MHz):

Carbon number	$\delta$ H (multiplicity, <i>J</i> in Hz)	$\delta$ C
1	6.86 (d, 8.4)	121.93
2	6.90 (d, 8.4)	110.67
3	-	149.18
4	-	145.07
<u>5</u> <sub>1</sub>	2.95 (dd, 3.3, 13.4)	36.51
<u>5</u> <sub>2</sub>	2.19 (dd, 13.4, 13.4)	
6	3.86 (m, 2.7, 3.3, 13.4)	68.60
7	4.28 (d, 2.7)	66.91
8	-	148.49
9	4.33 (d, 2.2)	53.13
10	4.53 (d, 2.2)	73.31
11	-	132.91
12	-	129.78
13	-	40.05
14	-	121.09
<u>15</u> <sub>1</sub>	1.99 (dd, 3.6, 12.5)	37.50
<u>15</u> <sub>2</sub>	1.84 (ddd, 4.7, 12.5, 12.5)	
<u>16</u> <sub>1</sub>	2.64 (dd, 4.7, 14.3)	40.85
<u>16</u> <sub>2</sub>	2.42 (ddd, 3.6, 14.3)	
3-OCH <sub>3</sub>	3.86 (s)	56.57
8-OCH <sub>3</sub>	3.69 (s)	57.06

b) Physico-chemical data of (1)

Mass Spectroscopy (ESI-TOF<sup>+</sup>): *m/z* 350.1 [M+H]<sup>+</sup>

Mass calculated for C<sub>18</sub>H<sub>24</sub>NO<sub>6</sub>: 350.1604

High Resolution MS (DCI<sup>+</sup>): *m/z* 350.1604 [M+H]<sup>+</sup>

UV ( $\lambda_{\text{max}}$  nm ( $\epsilon$ ), MeOH) : 282 (3318), 242<sub>sh</sub> (9926), 207 (57483)

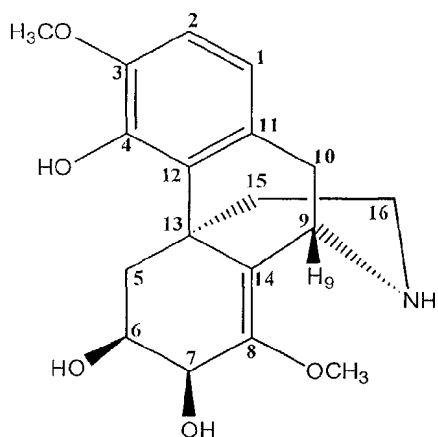
$[\alpha]_{\text{D}}^{20}$  - 46° (c 0.5, MeOH)

Circular Dichroism ( $\lambda_{\text{ext}}$  nm,  $\theta$ ) : (216, +66324), (227, -15949), (242, +16864)

(c = 1.15 × 10<sup>-3</sup> M, MeOH)

## 2) Compound (2)

[0059]



(2)

## a) NMR data of (2)

<sup>1</sup>H-NMR (300 MHz, CD<sub>3</sub>OD): δ 6.85 (d, 1H, *J* = 8.3 Hz), 6.62 (dd, 1H, *J* = 1.2, 8.3 Hz), 4.96 (dd, 1H, *J* = 1.7, 6.2 Hz), 4.39 (dd, 1H, *J* = 1.1, 3.1 Hz), 3.87 (ddd, 1H, *J* = 3.1, 3.9, 13.5 Hz), 3.84 (s, 3H), 3.78 (s, 3H), 3.31 (ddd, 3H, *J* = 1.2, 6.2, 19.1 Hz), 3.14 (dd, 1H, *J* = 4.6, 13.1 Hz), 3.10 (dd, 1H, *J* = 1.7, 19.1 Hz), 3.03 (ddd, 1H, *J* = 1.1, 3.9, 13.5 Hz), 2.84 (ddd, 1H, *J* = 4.1, 13.1, 13.2 Hz), 2.24 (dd, 1H, *J* = 13.5, 13.5 Hz), 2.19 (dd, 1H, *J* = 4.1, 13.2 Hz), 2.06 (ddd, 1H, *J* = 4.6, 13.2, 13.2 Hz)

<sup>13</sup>C-NMR (300 MHz, CD<sub>3</sub>OD): δ 33.53, 34.08, 36.0, 38.46, 40.67, 47.96, 56.22, 56.67, 65.38, 68.11, 111.30, 113.92, 119.48, 127.90, 128.56, 145.79, 148.09, 150.66

The NMR data of (2) were similar to those previously described in literature [1, 2].

## b) Physico-chemical data of (2)

Mass Spectroscopy (ESI-TOF<sup>+</sup>): *m/z* 334.1 [M+H]<sup>+</sup>

Mass calculated for C<sub>18</sub>H<sub>24</sub>NO<sub>5</sub>: 334.1654

High Resolution MS calculated for C<sub>18</sub>H<sub>23</sub>NO<sub>5</sub>: 334.1655

UV (MeOH) λ<sub>max</sub>nm (ε) : 282 (2856), 242<sub>sh</sub> (6984), 207 (59602)

[α]<sub>D</sub><sup>20</sup> - 143° (c 0.25, MeOH) cf. [3] [α]<sub>D</sub><sup>20</sup> - 137.4° (c 0.12, MeOH)

Circular Dichroism (λext<sub>nm</sub>, θ). (216, +63340), (227, -15231), (242, +16105)

(c = 1.20 x 10<sup>-3</sup> M, MeOH)

cf. [1] CD (λext<sub>nm</sub>, θ): (238, +62100) (c = 9.67 x 10<sup>-5</sup> M, MeOH)

## 3) Stereochemistry of compounds (1) and (2):

[0060] Specific rotation values of compound (2) and sinococuline were closely the same.

[0061] Compounds (1) and (2) showed the same Nuclear Overhauser Effect Spectroscopy (NOESY) correlations (cf. table 1) as sinococuline. Consequently compounds (1) and (2) have been assigned with the same relative configuration (6*S*, 7*S*, 9*R*, 13*S*) as sinococuline.

[0062] Moreover, compounds (1) and (2) exhibited similar CD spectra (positive maxima at 216 nm and 242 nm, negative maximum at 227 nm), in agreement with this of sinococuline described in literature, allowing us to conclude that the absolute configuration of compound (1) and compound (2) is (6*S*, 7*S*, 9*R*, 10*R*, 13*S*) and (6*S*, 7*S*, 9*R*, 13*S*) respectively.

Table 1:

NOESY (2D-NMR, 400 MHz, MeOH)		
	<u>Compound (1)</u>	<u>Compound (2)</u>
<u>Proton</u> <u>n°</u>	<u>Correlated protons</u>	<u>Correlated protons</u>
1	H10	H2, H10 <sub>1</sub> , H10 <sub>2</sub>
2	3-OCH <sub>3</sub>	H1, 3-OCH <sub>3</sub>
3	-	-
4	-	-
5 <sub>1</sub>	H15 <sub>1</sub> , H5 <sub>2</sub> , H6, H7	H5 <sub>2</sub> , H6
5 <sub>2</sub>	H5 <sub>1</sub> , H6	H5 <sub>1</sub> , H6
6	H15 <sub>2</sub> , H5 <sub>2</sub> , H5 <sub>1</sub> , H7	H5 <sub>1</sub> , H5 <sub>2</sub> , H7, H15 <sub>2</sub>
7	8-OCH <sub>3</sub> , H6	3-OCH <sub>3</sub> , H5 <sub>2</sub> , 8-OCH <sub>3</sub>
8	-	-
9	8-OCH <sub>3</sub> , H10	8-OCH <sub>3</sub> , H10 <sub>1</sub> , H10 <sub>2</sub>
10 <sub>1</sub>	H16 <sub>2</sub> , H9, H1 H16 <sub>2</sub> , H9, H1	H1, H9, H10 <sub>2</sub>
10 <sub>2</sub>		H1, H9, H10 <sub>1</sub>
11	-	-
12	-	-
13	-	-
14	-	-
15 <sub>1</sub>	H15 <sub>2</sub> , H16 <sub>2</sub> , H16 <sub>1</sub> , H5 <sub>1</sub>	H5 <sub>1</sub> , H15 <sub>2</sub> , H16 <sub>2</sub>
15 <sub>2</sub>	H15 <sub>1</sub> , H16 <sub>1</sub> , H6	H6, H15 <sub>1</sub> , H16 <sub>1</sub>
16 <sub>1</sub>	H15 <sub>1</sub> , H15 <sub>2</sub> , H16 <sub>2</sub>	H15 <sub>1</sub> , H15 <sub>2</sub> , H16 <sub>2</sub>
16 <sub>2</sub>	H15 <sub>1</sub> , H16 <sub>1</sub> , H10	H15 <sub>1</sub> , H16 <sub>1</sub>
3-OCH <sub>3</sub>	H2	H2
8-OCH <sub>3</sub>	H7, H9	H7

Example 5: *N*-methyl- 4, 6, 7, 10- tetrahydroxy- 8, 14, didehydro- 3, 8- dimethoxy-morphinan (3) C<sub>19</sub>H<sub>25</sub>NO<sub>6</sub>

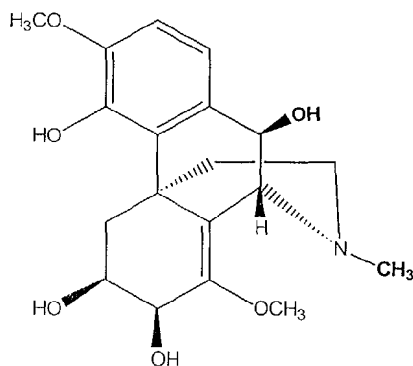
#### 1) Preparation of compound (3)

**[0063]** To a solution of (1) (10.9 mg, 0.031 mmol) in 1 mL MeOH was added 100 µl of a solution of formaldehyde (37 % in water). After stirring for 1Hour at room temperature, 3 mg of NaBH<sub>4</sub> was added and the mixture was stirred for additional 3 hours.

**[0064]** After removal of the solvent under reduced pressure, the residue was acidified with 1N HCl, then basified with 20% aqueous NH<sub>4</sub>OH and was further submitted to a silica gel column eluted with CH<sub>2</sub>Cl<sub>2</sub>-MeOH-NH<sub>4</sub>OH (85-15-1), leading to compound (3) as a white solid (8 mg, 0.022mmol), 71% yield.

## 2) Analytical data of (3)

[0065]



(3)

$^1\text{H-NMR}$  (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  6.90 (d, 1H,  $J = 8.4$  Hz), 6.83 (d, 1H,  $J = 8.4$  Hz), 4.70 (d, 1H,  $J = 2.2$  Hz), 4.30 (dd, 1H,  $J = 1.3, 3.4$  Hz), 4.21 (d, 1H,  $J = 2.2$  Hz), 3.88 (m, 1H,  $J = 3.4, 4.1, 13.8$  Hz), 3.87 (s, 3H), 3.70 (s, 3H), 2.98 (ddd, 1H,  $J = 1.3, 4.1, 13.8$  Hz), 2.51 (dd, 1H,  $J = 3.3, 12.4$  Hz), 2.47 (s, 3H), 2.26 (m, 1H,  $J = 2.9, 12.4, 12.9$  Hz), 2.18 (dd, 1H,  $J = 13.8, 13.8$  Hz), 1.95 (m, 2H,  $J = 2.9, 3.3, 12.9$  Hz)

Mass Spectroscopy (ESI-TOF $^+$ ):  $m/z$  364.1  $[\text{M}+\text{H}]^+$

Mass calculated for  $\text{C}_{19}\text{H}_{26}\text{NO}_6$ : 364.1760

High Resolution MS (DCI $^+$ ):  $m/z$  364.1764  $[\text{M}+\text{H}]^+$

$[\alpha]^{20}_{\text{D}} - 41^\circ$  (c 0.5, MeOH)

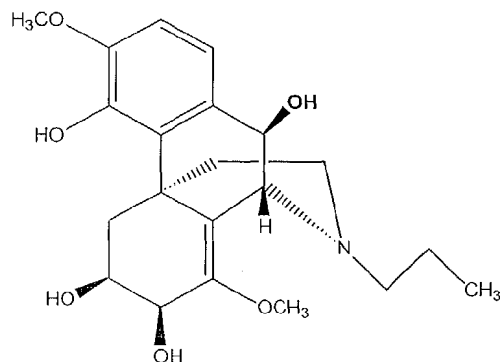
Example 6: *N*-propyl-4,6,7,10-tetrahydroxy-8,14-didehydro-3,8-dimethoxy-morphinan (4)  $\text{C}_{21}\text{H}_{29}\text{NO}_6$

## 1) Preparation of compound (4)

[0066] To a solution of (1) (3.05 mg, 0.0087 mmol) in 300  $\mu\text{l}$  MeOH, was added 1  $\mu\text{l}$ , 0.014 mmol of propionaldehyde and 0.6 mg, 0.0095 mmol of sodium cyanoborohydride  $\text{NaBH}_3\text{CN}$ . The mixture was stirring for 4 hours at room temperature.

[0067] After removal of the solvent under reduced pressure, the residue was acidified with HCl 1N, then basified with 20% aqueous  $\text{NH}_4\text{OH}$  and was submitted to a preparative TLC eluted twice with  $\text{CH}_2\text{Cl}_2$ -MeOH- $\text{NH}_4\text{OH}$  (90-10-1) to provide (4) as a white solid (2.3 mg, 0.0059 mmol), 68% yield.

## 2) Analytical data of (4)

**[0068]**

(4)

<sup>1</sup>H-NMR (300 MHz, CD<sub>3</sub>OD): δ 6.99 (d, 1H, *J* = 8.4 Hz), 6.96 (d, 1H, *J* = 8.4 Hz), 4.82 (d, 1H, *J* = 1.9 Hz), 4.72 (s, 1H), 4.37 (d, 1H, *J* = 3.3 Hz), 3.92 (m, 1H), 3.89 (s, 3H), 3.78 (s, 3H), 3.05 (m, 4H), 2.66 (m, 2H), 2.27 (dd, 1H, *J* = 13.5, 13.5 Hz), 2.11 (dd, 1H, *J* = 3.9, 12.3 Hz), 1.77 (m, 2H, *J* = 2.2, 7.3, 7.3 Hz), 1.02 (t, 3H, *J* = 7.3 Hz)

Mass Spectroscopy (ESI-TOF<sup>+</sup>): *m/z* 392.2 [M+H]<sup>+</sup>

Mass calculated for C<sub>21</sub>H<sub>30</sub>NO<sub>6</sub>: 392.2073

High Resolution MS (DCI<sup>+</sup>): *m/z* 392.2064 [M+H]<sup>+</sup>

[α]<sub>D</sub><sup>20</sup> -18° (c 0.1, MeOH)

Example 7: N-4-methoxybenzyl - 4, 6, 7, 10- tetrahydroxy- 8, 14, didehydro-3, 8- dimethoxy-morphinan (5) C<sub>26</sub>H<sub>31</sub>NO<sub>7</sub>

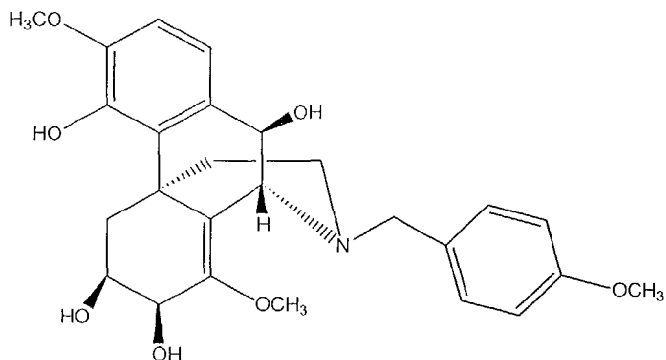
## 1) Preparation of compound (5):

**[0069]** To a solution of (1) (5.7 mg, 0.016 mmol) in 500 μl MeOH, was added 200 μl, 1.6 mmol of anisaldehyde. After stirring for 1Hour at room temperature, 3 mg of NaBH<sub>4</sub> was added and the mixture was stirred for additional 3 hours at room temperature.

**[0070]** After removal of the solvent under reduced pressure, the residue was acidified with HCl 1N, then basified with 20% aqueous NH<sub>4</sub>OH and was submitted to a preparative TLC, eluted twice with CH<sub>2</sub>Cl<sub>2</sub>-MeOH-NH<sub>4</sub>OH (90-10-1.5) to provide (5) as a white solid (3.0 mg, 0.0064mmol), 40% yield.

## 2) Analytical data of (5)

[0071]



(5)

<sup>1</sup>H-NMR (300 MHz, CD<sub>3</sub>OD): δ 7.47 (d, 2H, *J* = 8.6 Hz), 7.0 (d, 2H, *J* = 8.6 Hz), 6.98 (d, 1H, *J* = 8.4 Hz), 6.93 (d, 1H, *J* = 8.4 Hz), 4.88 (s, 1H), 4.71 (s, 1H), 4.40 (d, 1H, *J* = 2.8 Hz), 4.25 (s, 2H), 3.99 (m, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.77 (s, 3H), 3.06 (m, 2H, *J* = 3.6, 3.9, 12.5, 13.6 Hz), 2.75 (dd, 1H, *J* = 2.1, 12.5 Hz), 2.28 (ddd, 1H, *J* = 3.2, 13.6, 13.6 Hz), 2.20 (dd, 1H, *J* = 3.9, 13.2 Hz), 2.07 (dd, 1H, *J* = 2.1, 13.2 Hz)

MS (ESI-TOF<sup>+</sup>): *m/z* 470.2 [M+H]<sup>+</sup>

Mass calculated for C<sub>26</sub>H<sub>32</sub>NO<sub>7</sub>: 470.2179

High Resolution MS (DCI<sup>+</sup>): *m/z* 470.2170 [M+H]<sup>+</sup>

[α]<sub>D</sub><sup>20</sup> -12° (c 0.1, MeOH)

Example 8: *N*-4-hydroxybenzyl - 4, 6, 7, 10- tetrahydroxy- 8, 14, didehydro-3, 8- dimethoxy-morphinan (6) C<sub>25</sub>H<sub>29</sub>NO<sub>7</sub>

## 1) Preparation of compound (6)

[0072] To a solution of (1) (4.85 mg, 0.014 mmol) in 500 μl of MeOH was added 4-hydroxy-benzaldehyde (1.7 mg, 1 eq). After stirring for 4 hours at room temperature, 0.9 mg, 1 eq of NaBH<sub>3</sub>CN was added and the mixture was stirred for additional 3 hours at room temperature.

[0073] After removal of the solvent under reduced pressure, the residue was acidified with HCl 1N, then basified with 20% aqueous NH<sub>4</sub>OH and was submitted to a preparative TLC, eluted twice with CH<sub>2</sub>Cl<sub>2</sub>-MeOH-NH<sub>4</sub>OH (90-10-1) to provide (6) as a white solid (3.5 mg, 0.0077mmol), 55% yield.

## 2) Analytical data of (6)

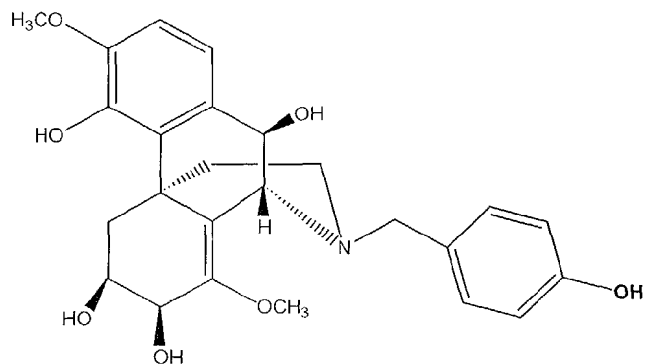
[0074]

5

10

15

20



(6)

<sup>1</sup>H-NMR (300 MHz, CD<sub>3</sub>OD): δ 7.29 (d, 2H, J = 8.6 Hz), 6.96 (d, 1H, J = 8.4 Hz), 6.91 (d, 1H, J = 8.4 Hz), 6.82 (d, 2H, J = 8.6 Hz), 4.79 (s, 1H), 4.57 (d, 1H, J = 1.6 Hz), 4.37 (d, 1H, J = 3.3 Hz), 4.01 (d, 2H, J = 8.1 Hz), 3.94 (ddd, 1H, J = 3.7, 3.7, 12.9 Hz), 3.87 (s, 3H), 3.72 (s, 3H), 3.02 (dd, 1H, J = 4.1, 13.5 Hz), 2.88 (d, 1H, J = 12.6 Hz), 2.59 (ddd, 1H, J = 3.7, 12.6, 12.6 Hz), 2.25 (dd, 1H, J = 13.5, 13.5 Hz), 2.12 (ddd, 1H, J = 4.5, 12.6, 12.6 Hz), 2.01 (d, 1H, J = 12.6 Hz)

Mass Spectroscopy (ESI-TOF<sup>+</sup>): *m/z* 456.2 [M+H]<sup>+</sup>

Mass calculated for C<sub>25</sub>H<sub>30</sub>NO<sub>7</sub>: 456.2022

High Resolution MS (DCI<sup>+</sup>): *m/z* 456.2018 [M+H]<sup>+</sup>

[α]<sub>D</sub><sup>20</sup> -10° (c 0.03, MeOH)

Example 9: *N*-4-bromobenzyl - 4, 6, 7, 10- tetrahydroxy- 8, 14, didehydro-3, 8- dimethoxy-morphinan (7) C<sub>25</sub>H<sub>28</sub>NO<sub>6</sub>Br

35

## 1) Preparation of compound (7)

[0075] To a solution of (1) (3.3 mg, 0.0095 mmol) in 300 μl MeOH was added 2 mg, 0.011 mmol of 4-bromo-benzaldehyde and 3 mg of NaBH<sub>3</sub>CN. The mixture was stirred 20 hours at room temperature.

[0076] After removal of the solvent under reduced pressure, the residue was acidified with HCl 1N, then basified with 20% aqueous NH<sub>4</sub>OH and was submitted to a preparative TLC eluted twice with CH<sub>2</sub>Cl<sub>2</sub>-MeOH-NH<sub>4</sub>OH (95-5-0.5) to provide (7) as a white solid (3.0 mg, 0.0058 mmol), 61% yield.

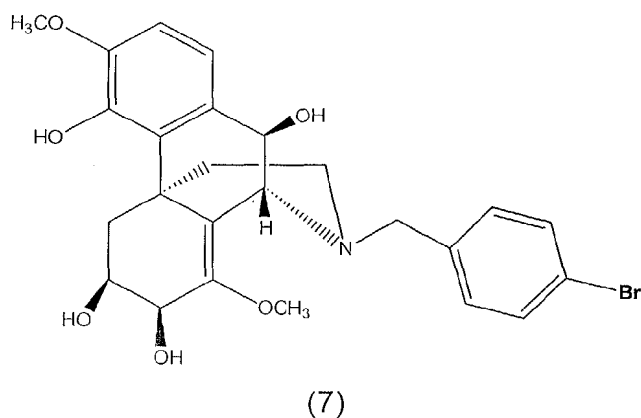
45

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55

## 2) Analytical data of (7)

[0077]



<sup>1</sup>H-NMR (300 MHz, CD<sub>3</sub>OD): δ 7.51 (d, 2H, *J* = 8.4 Hz), 7.35 (d, 2H, *J* = 8.4 Hz), 6.92 (d, 1H, *J* = 8.4 Hz), 6.88 (d, 1H, *J* = 8.4 Hz), 4.78 (d, 1H, *J* = 3.4 Hz), 4.74 (s, 2H), 4.73 (d, 1H, *J* = 2.2 Hz), 4.32 (d, 1H, *J* = 1, 3.4 Hz), 3.93 (ddd, 1H, *J* = 2.9, 3.4, 13.5 Hz), 3.88 (s, 3H), 3.68 (s, 3H), 3.0 (dd, 1H, *J* = 2.9, 13.5 Hz), 2.66 (m, 1H, *J* = 4.6, 12.1 Hz), 2.46 (ddd, 1H, *J* = 3.9, 12.1, 12.1 Hz), 2.21 (dd, 1H, *J* = 13.5, 13.5 Hz), 2.05 (ddd, 1H, *J* = 4.6, 12.6, 12.6 Hz), 1.94 (ddd, 1H, *J* = 1.9, 3.9, 12.6 Hz)

Mass Spectroscopy (ESI-TOF<sup>+</sup>): *m/z* 519.1 and 521.1 [M+H]<sup>+</sup>

Mass calculated for C<sub>25</sub>H<sub>29</sub>NO<sub>6</sub>Br: 519.1035 and 521.1055

High Resolution MS (DCI<sup>+</sup>): *m/z* 519.1037 and 521.1058 [M+H]<sup>+</sup>

[α]<sub>D</sub><sup>20</sup> -0.5° (c 0.5, MeOH)

Example 10: *N*-cyclopentyl- 4, 6, 7, 10- tetrahydroxy- 8, 14, didehydro- 3, 8-dimethoxy-morphinan (8) C<sub>23</sub>H<sub>31</sub>NO<sub>6</sub>

## 1) Preparation of compound (8)

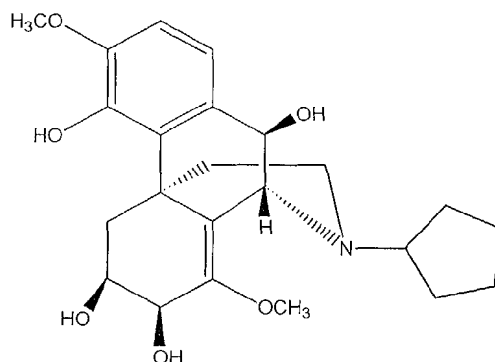
[0078] To a solution of (1) (4.95 mg, 0.014 mmol) in 300 μl of MeOH was added 2 μl, 0.023 mmol of cyclopentanone and 3 mg of NaBH<sub>3</sub>CN. The mixture was stirred for 6 hours at room temperature.

[0079] After removal of the solvent under reduced pressure, the residue was acidified with 1 N HCl, then basified with 20% aqueous NH<sub>4</sub>OH and was submitted to a preparative TLC eluted twice with CH<sub>2</sub>Cl<sub>2</sub>-MeOH-NH<sub>4</sub>OH (90-10-1) to provide (8) as a white solid (4.75 mg, 0.011 mmol), 81% yield.



## 2) Analytical data of (8)

[0080]



(8)

$^1\text{H-NMR}$  (300 MHz,  $\text{CD}_3\text{OD}$ ) :  $\delta$  6.98 (d, 1H,  $J = 8.4$  Hz), 6.94 (d, 1H,  $J = 8.4$  Hz), 4.83 (s, 1H), 4.71 (s, 1H), 4.37 (d, 1H,  $J = 2.7$  Hz), 3.91 (m, 1H), 3.88 (s, 3H), 3.78 (s, 3H), 3.21 (d, 1H,  $J = 12.6$  Hz), 3.05 (dd, 1H,  $J = 3.5, 14.3$  Hz), 2.27 (m, 2H,  $J = 12.6, 14.3$  Hz), 2.16 (m, 1H), 1.86 (m, 4H), 1.65 (m, 6H)

Mass Spectroscopy (ESI-TOF $^+$ ):  $m/z$  418.2  $[\text{M}+\text{H}]^+$

Mass calculated for  $\text{C}_{23}\text{H}_{32}\text{NO}_6$ : 418.2230

High Resolution MS (DCI $^+$ ):  $m/z$  418.2239  $[\text{M}+\text{H}]^+$

$[\alpha]_D^{20}$  -30° (c 0.03, MeOH)

Evaluation of the biological activity of compounds

• Materials and Methods

- • *In vitro* evaluation of the antimalarial activity of plant extracts and pure compounds (1)-(8)

**A-** Murine model: Hepatic Stage of *Plasmodium yoelii yoelii* 265BY.

**[0081]** Primary cultures of mouse hepatocytes were isolated from livers of Swiss mice which were 6 to 8 weeks old by perfusion with collagenase (1 g /L) and purified through a 60% Percoll gradient.

**[0082]** The hepatocytes were cultured in sterile chambers (Lab-Tek) at the rate of 90,000 cells in 0.3 mL of complete culture medium as defined thereafter per well and incubated overnight at 37°C under a 4%  $\text{CO}_2$  atmosphere. The hepatocytes' complete culture medium was Williams' E culture medium supplemented with 10% of decompartmented foetal calf serum, 1% glutamine, 1% Na pyruvate, 1% of a mixture of insulin, transferrine and selenium, 1% of non-essential amino-acids and with an antibiotic mixture comprising 1% of a solution of penicillin and streptomycin, 1% augmentin and 2.5 pg/ml flucytosine.

**[0083]** The parasites infecting the hepatocytes; i.e. the sporozoites, were recovered by dissection of infected anophelines mosquitoes salivary glands.

**[0084]** After grinding them in a Potter grinder with culture medium, the suspension was filtered on a 40  $\mu\text{m}$  mesh filter and centrifuged (15,000 rpm) at 4°C for 2 mn. The pellet was reintroduced in the complete culture medium. The sporozoites were then counted in a "Cell Vu" counter (CML) and the concentration was adjusted at 100,000 sporozoites per 70  $\mu\text{L}$ , concentration needed for infection.

**[0085]** One day after, the hepatocytes were infected by the above sporozoites from salivary glands of infected anophelines mosquitoes at the rate of 100,000 sporozoites per well in the presence or absence of the product to be tested. When the infection had appeared, the medium was supplemented with  $10^{-7}$  M dexamethasone. The products were first solubilized in DMSO.

[0086] The infected hepatocytes were washed and fed with culture medium containing the test product, 3 hours and then 24 hours after the infection.

[0087] The cultures were stopped by treatment with a cold fixative methanol solution, 48 hours after the infection.

[0088] Parasites obtained in schizont stage were immunomarked firstly, with a serum isolated from BALB/c mice immunized with a recombinant fragment from the N-terminal of the recombinant protein 172 that reacted against HSP70 of *Plasmodium* [4] and secondly with an antibody- a mouse anti-immunoglobulin- conjugated to FITC. Evans Blue and DAPI, a nucleic acid marker, were added to the hepatocytes simultaneously. The schizonts were then counted with a fluorescent microscope.

[0089] Extracts and purified products exhibiting a significant inhibition effect on *P. yoelii yoelii* 265BY growth in murine hepatocytes were tested on human hepatocyte cultures infected with *P. falciparum* NF54.

B - Human model: Hepatic stage of *Plasmodium falciparum* NF54

[0090] Human hepatocytes were obtained by enzymatic perfusion of fragments of liver of human adults which were submitted to partial hepatectomy.

[0091] The hepatocytes were infected at 24 hours of their culture by sporozoites obtained from salivary glands of *P. falciparum* infecting anopheles mosquitoes *stephensi* (Lab. of Pr. W. Eling, Univ. Of Nijmegen, NL).

[0092] The culture medium containing the test product was changed 3 hours after infection, and then renewed at least, each 24 hours during the experiment.

[0093] The cultures were fixed 5 days after infection, with the same method as described for the murine hepatocytes [5].

[0094] The  $CI_{50}$  value (concentration causing 50% of inhibition in the number of parasites compared to controls wells, non treated) is the mean value of 3 independent evaluations through StatView SI Graphics.

• • *In vitro* evaluation of the cytotoxicity of plant extracts and pure compounds (1)-(8)

[0095] The cytotoxicity assays were carried out in 96-well microliter plates, in triplicate, against human carcinoma KB cell line (ATCC CCL-17) ( $10^4$  cells /mL in DMEM medium) and human colon tumor HT29 cell line (ATCC HTB-38) ( $10^5$  cells /mL in RPMI 1640 medium), both supplemented with 10% foetal calf serum, L-glutamine (2mM), penicillin G (100 UI/mL), streptomycin (100 µg/mM) and gentamycin (10 µg/mL).

[0096] Stock solutions of testing compounds were prepared in H<sub>2</sub>O/DMSO (9/1).

[0097] After an incubation of 72 hours at 37°C under a 5% CO<sub>2</sub> atmosphere, 0.02% of neutral red in PBS was added.

[0098] 24 hours later, culture media were eliminated and cell membranes were lysed by addition of 1 % of SDS in water.

[0099] Cell growth was estimated by colorimetric measurement of stained living cells, incorporating neutral red. Optical density was determined at 540 nm on a Titertek Multiskan photometer.

[0100] The  $CI_{50}$  value was defined as the concentration of compound necessary to inhibit the cell growth to 50% of the control.

• Results

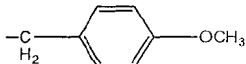
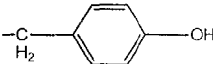

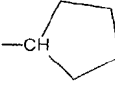
[0101] Table 2 represents *in vitro* activities of natural compounds (1) and (2) and N-substituted derivatives of (1), compounds (3)-(8), on growth inhibition of *P. yoelii yoelii* 265 BY in murine hepatocytes and their cytotoxicity on human tumor cell lines.

[0102] In Table 2, R represents the group introduced on the nitrogen atom by chemical modification of (1) as defined in formula I.

[0103] The selectivity index ( = ratio between  $CI_{50}$  of cytotoxicity and  $CI_{50}$  of anti-malarial activity) that was calculated, should the real effect of the compounds on *Plasmodium* parasites with respect to mammalian cells.

[0104] Primaquine and atovaquone were tested as references for their inhibiting activities on hepatic stage of *Plasmodium*, while 5-fluorouracile and vinblastine were tested as references for their cytotoxic activities on KB and HT29 cells.

Table 2

Compound - R <sub>10</sub>	CI <sub>50</sub> activity <i>P. yoelii</i> 265BY in µg/mL, (µM)	CI <sub>50</sub> cytotoxicity KB in µg/mL, (µM)	CI <sub>50</sub> cytotoxicity HT29 in µg/mL, (µM)	Selectivity index CI <sub>50</sub> KB/ CI <sub>50</sub> <i>yoelii</i>
(1)	1.08	1.99 ± 1.3	22.0 ± 12.5	1.8
- H	[0.15 -0.32]	5.7	63.0	
	3.1			
(2)	1.50	1.30 ± 0.6	12.24 ± 8.59	0.9
- H	[0.25- 0.45]	3.9	36.8	
	4.5			
(3)	2.09	32.31 ± 7.1	> 80	15.5
- CH <sub>3</sub>	[0.87 -0.67]	89.0	> 220	
	5.8			
(4)	4.93	40.37 ± 13.9	> 80	8.2
- CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>3</sub>	[0.69 -0.60]	103.2	> 204	
	12.6			
(5)	11.4	21.23 ± 6.2	> 80	1.9
	[0.75 -0.64]	45.3	> 170	
	24.2			
(6)	6.44	9.53 ± 3.3	48.96 ± 1.8	1.5
	[0.92 -0.80]	20.9	107.6	
	14.2			
(7)	2.19	15.81 ± 6.4	> 80	7.3
	[0.92 -0.71]	30.5	> 154	
	4.2			
(8)	1.46	27.64 ± 9.0	> 80	18.9
	[1.01 -0.89]	66.28	> 192	
	3.5			
primaquine	0.16	2.0 ± 0.9	20.12 ± 2.1	11.7
	[0.11 -0.28]	7.0	70.51	
	0.6			
Atovaquone	0.021	18.60 ± 8.7	44.60 ± 10.3	891.2
	[.003-.009]	50.8	122	
	0.057			
5-fluorouracile	/	0.047 ± 0.03	54.88 ± 2.4	/
		0.36	422	

- R	CI <sub>50</sub> activity <i>P. yoelii</i> 265BY in µg/mL, (µM)	CI <sub>50</sub> cytotoxicity KB in µg/mL, (µM)	CI <sub>50</sub> cytotoxicity HT29 in µg/mL, (µM)	Selectivity index CI <sub>50</sub> KB/ CI <sub>50</sub> <i>yoelii</i>
Vinblastine	/	0.018 ± 0.006 0.022	0.0080 ± 0.004 0.0099	/

[0105] Table 3 represents *in vitro* activities of natural compounds (1) and (2), on *P. falciparum* NF54 in human hepatocytes.

Table 3

	CI <sub>50</sub> activity <i>P. falciparum</i> NF54 in µg/mL, (µM)
(1)	1.51 [1.01 - 0.34] 4.3
(2)	3.36 [1.35 - 0.92] 10.1

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## [0106]

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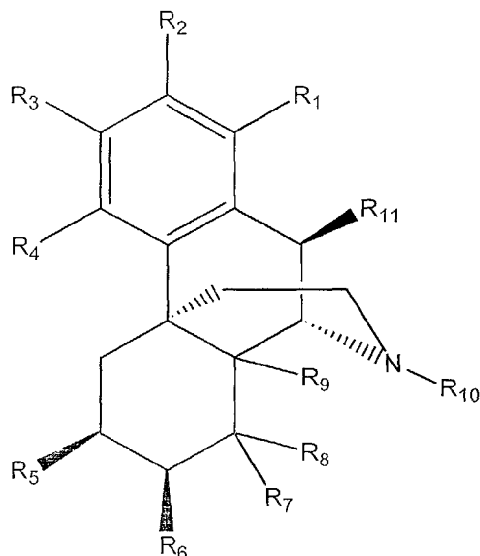
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[5] Mazier D., Beaudoin R.L., Mellouk S., Druilhe P., Texier B., Trosper J., Miltgen F., Landau I., Paul C., Brandicourt O., *et al.* Complete development of hepatic stages of *Plasmodium falciparum* *in vitro*. *Science*, 1985, 227(4685), 440-2.

## Claims

1. Chemical compound having the following formula

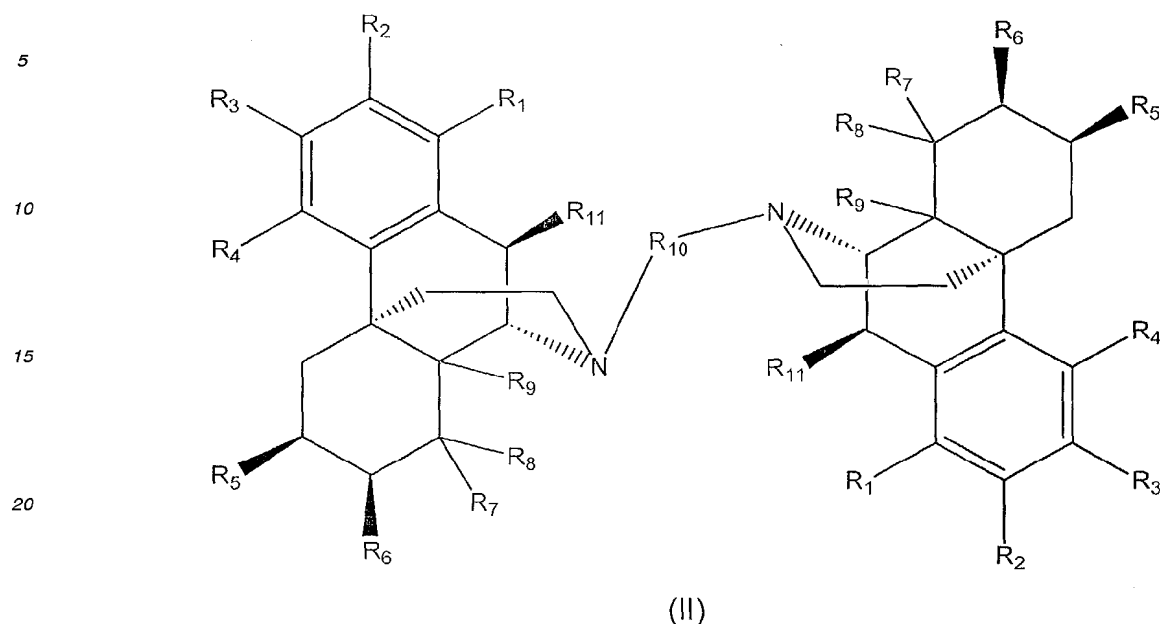


(I)

wherein

- $R_1$  is H or  $OC_iH_{2i+1}$  with  $i$  between 0 and 6;
- $R_2$  is H or  $OC_jH_{2j+1}$  with  $j$  between 0 and 6;
- $R_3$  is H or  $OC_kH_{2k+1}$  with  $k$  between 0 and 6;
- $R_4$  is H or OH;
- $R_5$  is OH or  $OC_mH_{2m+1}$  with  $m$  between 1 and 6 or an acetoxy group or an oxo group;
- $R_6$  is OH or  $OC_nH_{2n+1}$  with  $n$  between 1 and 6 or an acetoxy group or an oxo group;
- $R_7$  is  $OC_pH_{2p+1}$  with  $p$  between 0 and 6;
- $R_8$  and  $R_9$  may be similar or different and represent H or  $OC_qH_{2q+1}$  with  $q$  between 0 and 6, or Hal where Hal is Cl, Br, F or I, or form together a covalent bond whereby the bond is a double bond or an ether bond (epoxy group);  
 $R_{10}$  is H or  $C_rH_{2r+1}$  with  $r$  between 1 and 12 or an unsaturated alkyl group or a cycloalkyl group (tri- to hexa-) unsaturated or not, with or without heteroatoms; or an aromatic or a polycyclic aromatic group with or without heteroatoms; or  $C_sH_{2s}-A$  with  $s$  between 1 and 12 and whereby A is a saturated or (tri- to hexa-) unsaturated cycloalkyl group, with or without heteroatoms or an aromatic or a polycyclic aromatic group with or without heteroatoms or A is  $C_6H_5-t-(Hal)_t$  with  $t$  between 1 to 5 or  $C_6H_5-u-(O-C_vH_{2v+1})_u$  with  $u$  between 1 to 5 and  $v$  between 0 and 6; or  $R_{10}$  represents two substituents similar or different rendering the nitrogen atom quaternized, or an oxygen atom (nitron group) and in which case the bond between the nitrogen atom and  $C_9$  is a double bond; or  $R_{10}$  represents  $CH_2CH_2[OCH_2CH_2]_wOCH_2CH_2-B$  with  $w$  between 0 and 10 and where B is OH, O-D or NH-D where D is a  $C_1-C_{12}$  alkyl group bearing an electrophilic function such as an isothiocyanate;
- $R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with  $x$  between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group; or  $R_{10}$  and  $R_{11}$  may represent an isoalkylidene group;

or



wherein  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  are defined as above,

- $R_4$  is H or OH or  $OC_lH_{2l+1}$  with  $l$  between 2 and 6;
- $R_{10}$  is  $C_yH_{2y}$  with  $y$  between 1 and 12 or  $CH_2CH_2[OCH_2CH_2]_zOCH_2CH_2$  with  $z$  between 0 and 10; and

$R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with  $x$  between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group;

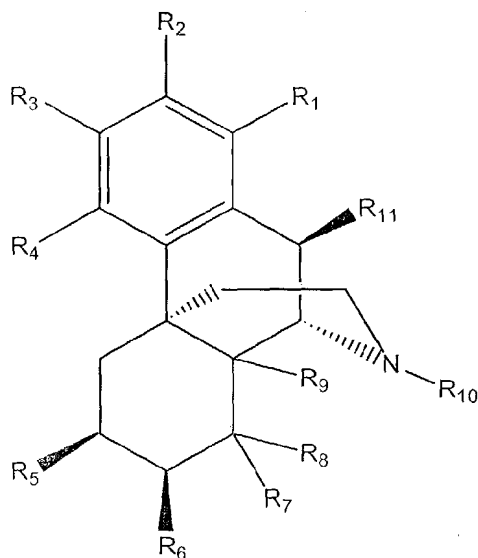
and all stereoisomers and optical isomers thereof,

with the proviso that in formula (I) (1)  $R_2$  is not H or alkoxy when  $R_3$  is OH or alkoxy and  $R_4$  is H or OH and  $R_5$  and  $R_6$  are OH or acyloxy and  $R_7$  is  $OCH_3$  and  $R_8$  and  $R_9$  represent a double bond and  $R_1$  is H; and (2)  $R_1$  is not H when  $R_2$  is H and  $R_3$  and  $R_7$  are  $OCH_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are OH and  $R_8$  and  $R_9$  represent a double bond and  $R_{10}$  is H.

Particularly, the invention concerns the compounds wherein  $R_{10}$  is OH.

2. Chemical compound of claim 1, wherein  $R_{11}$  is OH.
3. Chemical compound of claim 1 or 2, wherein  $R_8$  and  $R_9$  form a covalent bond.
4. Chemical compound of one of claim 1 to 3, wherein  $R_{11}$  is OH and  $R_1$  and  $R_2$  are H.
5. Chemical compound of claim 1 to 4, wherein  $R_{11}$  is H or  $C_rH_{2r}$ , A with  $r$  between 1 and 12 and whereby A is H or a cycle  $C_sH_{2s-1}$  with  $s$  between 3 and 6 or an aromatic cycle or an aromatic polycycle or an aromatic cycle substituted as  $C_6H_{5-t}(Hal)_t$  with  $t$  between 1 to 5 and where Hal is Cl, Br, F or I, or as  $C_6H_{5-u}(O-C_vH_{2v+1})_u$  with  $u$  between 1 to 5 and  $v$  between 0 to 6, or as  $C_6H_{5-w}(NH_2)_w$  with  $w$  between 1 to 2; or  $R_{10}$  represents two substituents similar or different rendering the nitrogen atom quaternized, or an oxygen atom; or  $R_{10}$  represents  $CH_2-CH_2-[O-CH_2-CH_2]_xO-CH_2-CH_2-B$  with  $x$  between 0 and 10 and whereby B is H or OH or  $NH_2$  or  $N=C=S$ .
6. Chemical compound of claim 1, selected from the group comprising 4, 6, 7, 10- tetrahydroxy- 8, 14- didehydro- 3, 8-dimethoxymorphinan and *N*-methyl-, *N*-propyl-, *N*-4-methoxybenzyl-, *N*-4-hydroxybenzyl-, *N*-4-bromobenzyl- or *N*-cyclopentyl- - 4, 6, 7, 10-tetrahydroxy- 8, 14- didehydro- 3, 8- dimethoxy-morphinan.
7. Chemical compound of claim 6 having the optical configuration (6R, 7S, 9R, 10R, 13S).

## 8. Medicament having the following formula

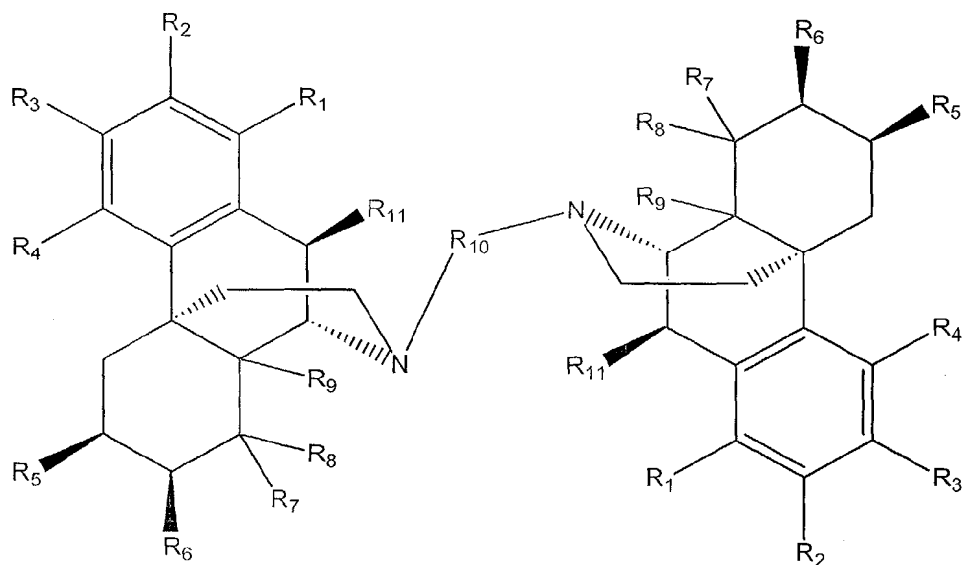


(I)

wherein

- $R_1$  is H or  $OC_iH_{2i+1}$  with  $i$  between 0 and 6;
- $R_2$  is H or  $OC_jH_{2j+1}$  with  $j$  between 0 and 6;
- $R_3$  is H or  $OC_kH_{2k+1}$  with  $k$  between 0 and 6;
- $R_4$  is H or OH;
- $R_5$  is OH or  $OC_mH_{2m+1}$  with  $m$  between 1 and 6 or an acetoxy group or an oxo group;
- $R_6$  is OH or  $OC_nH_{2n+1}$  with  $n$  between 1 and 6 or an acetoxy group or an oxo group;
- $R_7$  is  $OC_pH_{2p+1}$  with  $p$  between 0 and 6;
- $R_8$  and  $R_9$  may be similar or different and represent H or  $OC_qH_{2q+1}$  with  $q$  between 0 and 6, or Hal where Hal is Cl, Br, F or I, or form together a covalent bond whereby the bond is a double bond or an ether bond (epoxy group);
- $R_{10}$  is H or  $C_rH_{2r+1}$  with  $r$  between 1 and 12 or an unsaturated alkyl group or a cycloalkyl group (tri- to hexa-) unsaturated or not, with or without heteroatoms; or an aromatic or a polycyclic aromatic group with or without heteroatoms; or  $C_sH_{2s}-A$  with  $s$  between 1 and 12 and whereby A is a saturated or (tri- to hexa-) unsaturated cycloalkyl group, with or without heteroatoms or an aromatic or a polycyclic aromatic group with or without heteroatoms or A is  $C_6H_5-t-(Hal)_t$  with  $t$  between 1 to 5 or  $C_6H_5-u-(O-C_vH_{2v+1})_u$  with  $u$  between 1 to 5 and  $v$  between 0 and 6; or  $R_{10}$  represents two substituents similar or different rendering the nitrogen atom quaternized, or an oxygen atom (nitron group) and in which case the bond between the nitrogen atom and  $C_9$  is a double bond; or  $R_{10}$  represents  $CH_2CH_2[OCH_2CH_2]_wOCH_2CH_2-B$  with  $w$  between 0 and 10 and where B is OH, O-D or NH-D where D is a  $C_1-C_{12}$  alkyl group bearing an electrophilic function such as an isothiocyanate;

- $R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with x between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group; or  $R_{10}$  and  $R_{11}$  may represent an isoalkylidene group; or



(II)

wherein  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  are defined as above,

- $R_4$  is H or OH or  $OC_lH_{2l+1}$  with l between 2 and 6;
- $R_{10}$  is  $C_yH_{2y}$  with y between 1 and 12 or  $CH_2CH_2[OCH_2CH_2]_zOCH_2CH_2$  with z between 0 and 10; and

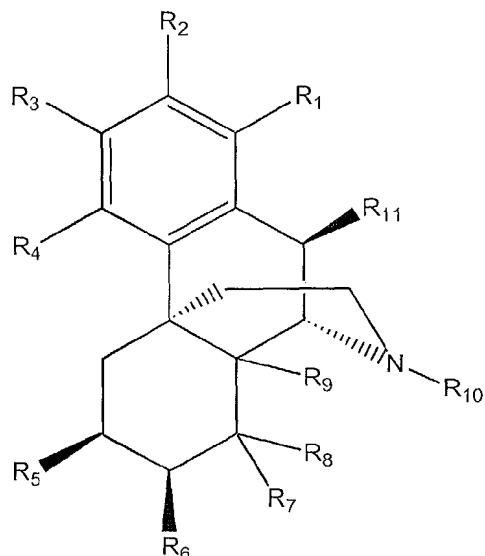
$R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with x between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group ;

and all stereoisomers and optical isomers thereof,

with the proviso that in formula (I) (1)  $R_2$  is not H or alkoxy when  $R_3$  is OH or alkoxy and  $R_4$  is H or OH and  $R_5$  and  $R_6$  are OH or alkoxy and  $R_7$  is  $OCH_3$  and  $R_8$  and  $R_9$  represent a double bond and  $R_1$  is H; and (2)  $R_1$  is not H when  $R_2$  is H and  $R_3$  and  $R_7$  are  $OCH_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are OH and  $R_8$  and  $R_9$  represent a double bond and  $R_{10}$  is H.

## 9. Anti-malarial compound of formula

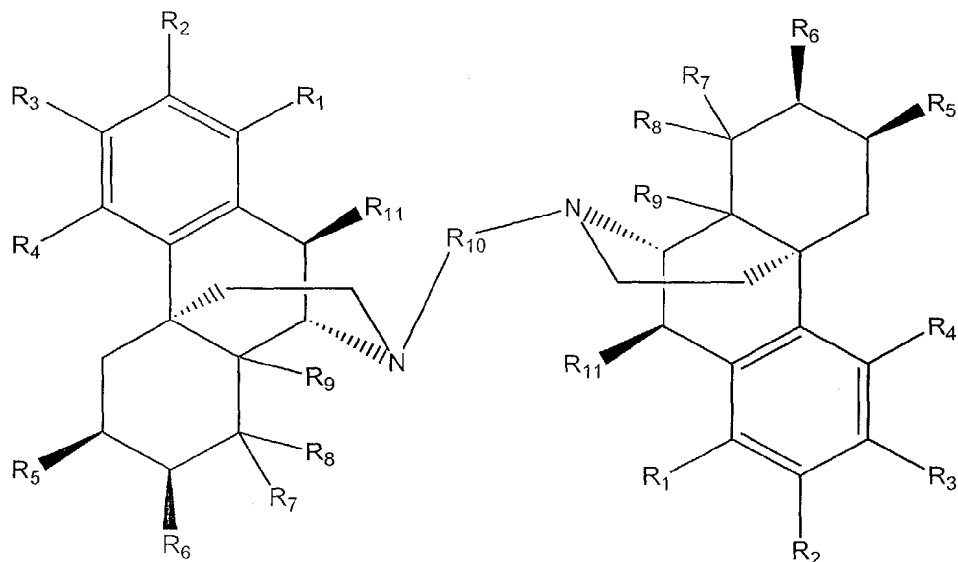




(I)

wherein

- $R_1$  is H or  $OC_iH_{2i+1}$  with  $i$  between 0 and 6;
- $R_2$  is H or  $OC_jH_{2j+1}$  with  $j$  between 0 and 6;
- $R_3$  is H or  $OC_kH_{2k+1}$  with  $k$  between 0 and 6;
- $R_4$  is H or OH;
- $R_5$  is OH or  $OC_mH_{2m+1}$  with  $m$  between 1 and 6 or an acetoxy group or an oxo group;
- $R_6$  is OH or  $OC_nH_{2n+1}$  with  $n$  between 1 and 6 or an acetoxy group or an oxo group;
- $R_7$  is  $OC_pH_{2p+1}$  with  $p$  between 0 and 6;
- $R_8$  and  $R_9$  may be similar or different and represent H or  $OC_qH_{2q+1}$  with  $q$  between 0 and 6, or Hal where Hal is Cl, Br, F or I, or form together a covalent bond whereby the bond is a double bond or an ether bond;
- $R_{10}$  is H or  $C_rH_{2r+1}$  with  $r$  between 1 and 12 or an unsaturated alkyl group or a cycloalkyl group (tri- to hexa-) unsaturated or not, with or without heteroatoms; or an aromatic or a polycyclic aromatic group with or without heteroatoms; or  $C_sH_{2s}-A$  with  $s$  between 1 and 12 and whereby A is a saturated or (tri- to hexa-) unsaturated cycloalkyl group, with or without heteroatoms or an aromatic or a polycyclic aromatic group with or without heteroatoms or A is  $C_6H_{5-t}-(Hal)_t$  with  $t$  between 1 to 5 or  $C_6H_{5-u}-(O-C_vH_{2v+1})_u$  with  $u$  between 1 to 5 and  $v$  between 0 and 6; or  $R_{10}$  represents two substituents similar or different rendering the nitrogen atom quaternized, or an oxygen atom (nitron group) and in which case the bond between the nitrogen atom and  $C_9$  is a double bond; or  $R_{10}$  represents  $CH_2CH_2[OCH_2CH_2]_wOCH_2CH_2-B$  with  $w$  between 0 and 10 and where B is OH, O-D or NH-D where D is a  $C_1-C_{12}$  alkyl group bearing an electrophilic function such as an isothiocyanate;
- $R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with  $x$  between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group; or  $R_{10}$  and  $R_{11}$  may represent an isoalkylidene group; or



(II)

wherein  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  are defined as above,

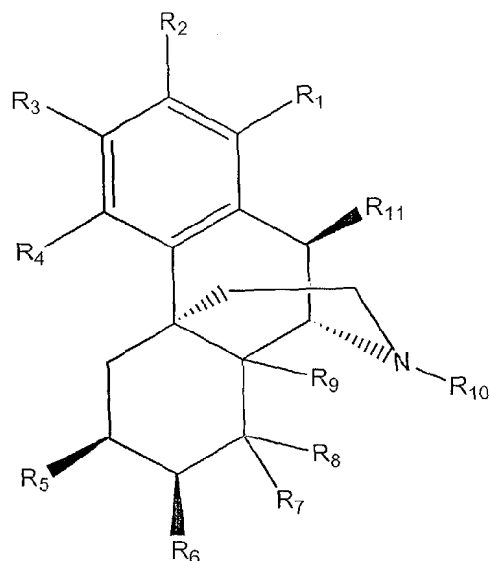
- $R_4$  is H or OH or  $OC_1H_{2l+1}$  with  $l$  between 2 and 6;
- $R_{10}$  is  $C_yH_{2y}$  with  $y$  between 1 and 12 or  $CH_2CH_2[OCH_2CH_2]_zOCH_2CH_2$  with  $z$  between 0 and 10; and

$R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with  $x$  between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group ;

and all stereoisomers and optical isomers thereof,

with the proviso that in formula (I) (1)  $R_2$  is not H or alkoxy when  $R_3$  is OH or alkoxy and  $R_4$  is H or OH and  $R_5$  and  $R_6$  are OH or alkoxy and  $R_7$  is  $OCH_3$  and  $R_8$  and  $R_9$  represent a double bond and  $R_1$  is H; and (2)  $R_1$  is not H when  $R_2$  is H and  $R_3$  and  $R_7$  are  $OCH_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are OH and  $R_8$  and  $R_9$  represent a double bond and  $R_{10}$  is H.

#### 10. Use of compound of formula I

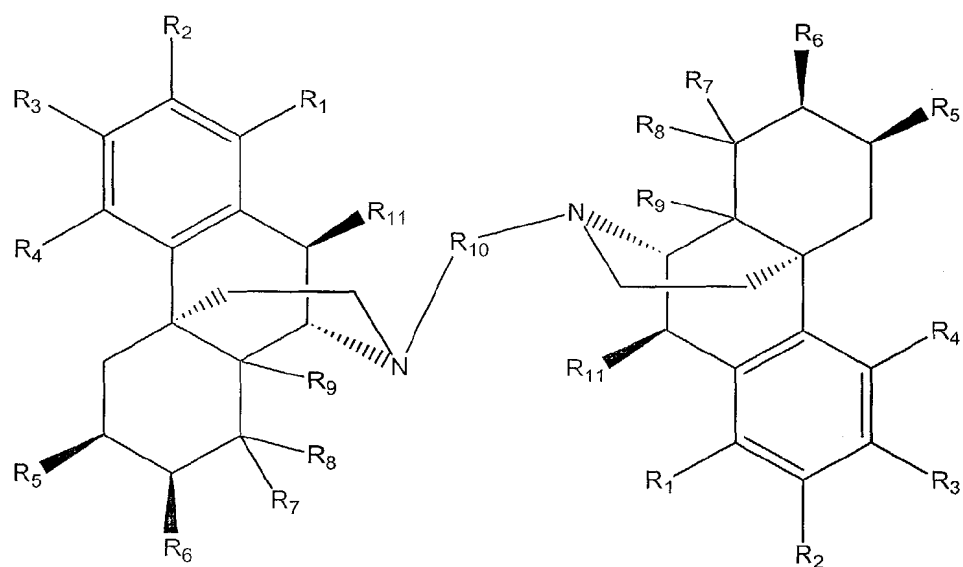


(I)

wherein

- $R_1$  is H or  $OC_iH_{2i+1}$  with  $i$  between 0 and 6;
- $R_2$  is H or  $OC_jH_{2j+1}$  with  $j$  between 0 and 6;
- $R_3$  is H or  $OC_kH_{2k+1}$  with  $k$  between 0 and 6;
- $R_4$  is H or OH;
- $R_5$  is OH or  $OC_mH_{2m+1}$  with  $m$  between 1 and 6 or an acetoxy group or an oxo group;
- $R_6$  is OH or  $OC_nH_{2n+1}$  with  $n$  between 1 and 6 or an acetoxy group or an oxo group;
- $R_7$  is  $OC_pH_{2p+1}$  with  $p$  between 0 and 6;
- $R_8$  and  $R_9$  may be similar or different and represent H or  $OC_qH_{2q+1}$  with  $q$  between 0 and 6, or Hal where Hal is Cl, Br, F or I, or form together a covalent bond whereby the bond is a double bond or an ether bond;
- $R_{10}$  is H or  $C_rH_{2r+1}$  with  $r$  between 1 and 12 or an unsaturated alkyl group or a cycloalkyl group (tri- to hexa-) unsaturated or not, with or without heteroatoms; or an aromatic or a polycyclic aromatic group with or without heteroatoms; or  $C_sH_{2s}-A$  with  $s$  between 1 and 12 and whereby A is a saturated or (tri- to hexa-) unsaturated cycloalkyl group, with or without heteroatoms or an aromatic or a polycyclic aromatic group with or without heteroatoms or A is  $C_6H_5-t-(Hal)_t$  with  $t$  between 1 to 5 or  $C_6H_5-u-(O-C_vH_{2v+1})_u$  with  $u$  between 1 to 5 and  $v$  between 0 and 6; or  $R_{10}$  represents two substituents similar or different rendering the nitrogen atom quaternized, or an oxygen atom (nitron group) and in which case the bond between the nitrogen atom and  $C_9$  is a double bond; or  $R_{10}$  represents  $CH_2CH_2[OCH_2CH_2]_wOCH_2CH_2-B$  with  $w$  between 0 and 10 and where B is OH, O-D or NH-D where D is a  $C_1-C_{12}$  alkyl group bearing an electrophilic function such as an isothiocyanate;
- $R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with  $x$  between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group; or  $R_{10}$  and  $R_{11}$  may represent an isoalkylidene group;

or



(II)

wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub> and R<sub>9</sub> are defined as above,

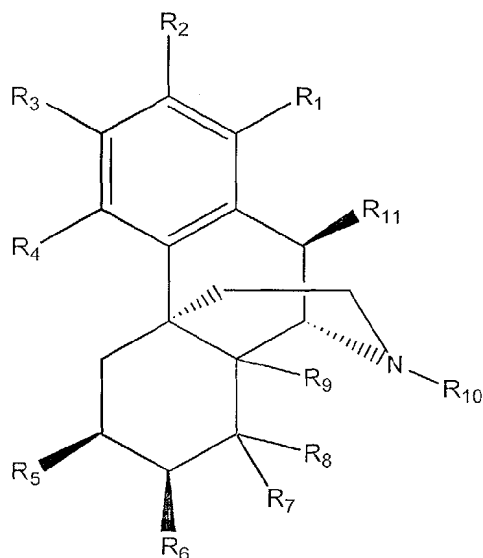
- R<sub>4</sub> is H or OH or OC<sub>l</sub>H<sub>2l+1</sub> with l between 2 and 6;
- R<sub>10</sub> is C<sub>y</sub>H<sub>2y</sub> with y between 1 and 12 or CH<sub>2</sub>CH<sub>2</sub>[OCH<sub>2</sub>CH<sub>2</sub>]<sub>z</sub>OCH<sub>2</sub>CH<sub>2</sub> with z between 0 and 10; and

R<sub>11</sub> is H or OH or OC<sub>x</sub>H<sub>2x+1</sub> with x between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group ;

all stereoisomers and optical isomers thereof,

and their pharmaceutically acceptable salts in the preparation of a pharmaceutical composition useful in an anti-malarial prophylactic or curative treatment.

#### 11. Use of compound of formula I

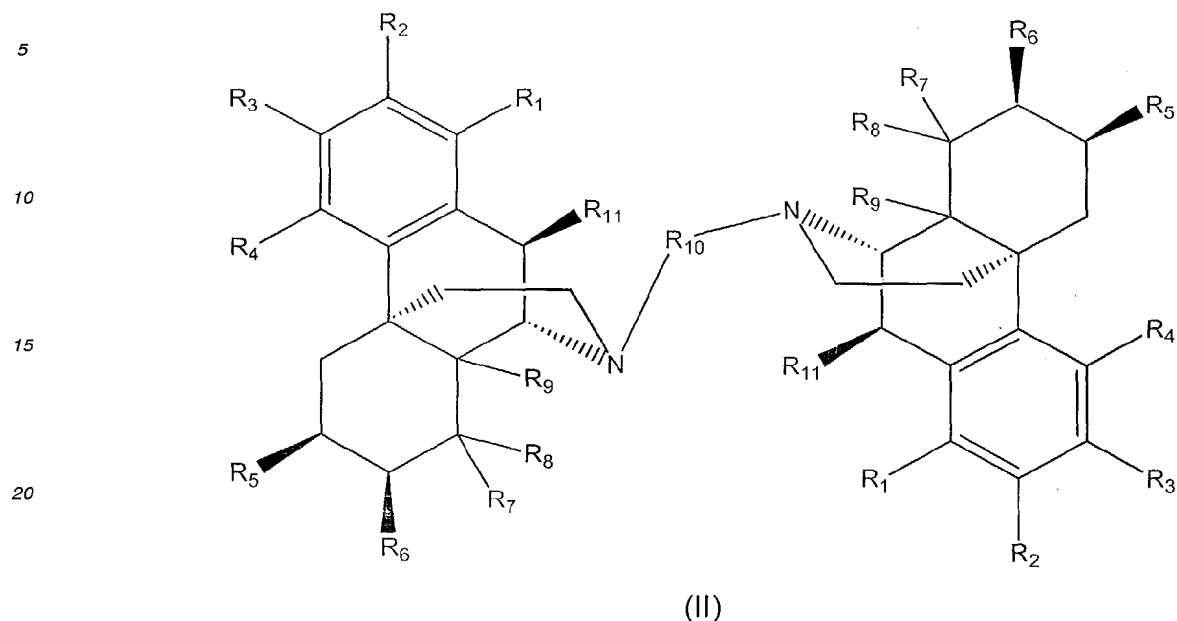


(I)

wherein

- $R_1$  is H or  $OC_iH_{2i+1}$  with  $i$  between 0 and 6;
- $R_2$  is H or  $OC_jH_{2j+1}$  with  $j$  between 0 and 6;
- $R_3$  is H or  $OC_kH_{2k+1}$  with  $k$  between 0 and 6;
- $R_4$  is H or OH;
- $R_5$  is OH or  $OC_mH_{2m+1}$  with  $m$  between 1 and 6 or an acetoxy group or an oxo group;
- $R_6$  is OH or  $OC_nH_{2n+1}$  with  $n$  between 1 and 6 or an acetoxy group or an oxo group;
- $R_7$  is  $OC_pH_{2p+1}$  with  $p$  between 0 and 6;
- $R_8$  and  $R_9$  may be similar or different and represent H or  $OC_qH_{2q+1}$  with  $q$  between 0 and 6, or Hal where Hal is Cl, Br, F or I, or form together a covalent bond whereby the bond is a double bond or an ether bond;
- $R_{10}$  is H or  $C_rH_{2r+1}$  with  $r$  between 1 and 12 or an unsaturated alkyl group or a cycloalkyl group (tri- to hexa-) unsaturated or not, with or without heteroatoms; or an aromatic or a polycyclic aromatic group with or without heteroatoms; or  $C_sH_{2s}-A$  with  $s$  between 1 and 12 and whereby A is a saturated or (tri- to hexa-) unsaturated cycloalkyl group, with or without heteroatoms or an aromatic or a polycyclic aromatic group with or without heteroatoms or A is  $C_6H_5-t-(Hal)_t$  with  $t$  between 1 to 5 or  $C_6H_5-u-(O-C_vH_{2v+1})_u$  with  $u$  between 1 to 5 and  $v$  between 0 and 6; or  $R_{10}$  represents two substituents similar or different rendering the nitrogen atom quaternized, or an oxygen atom (nitron group) and in which case the bond between the nitrogen atom and  $C_9$  is a double bond; or  $R_{10}$  represents  $CH_2CH_2[OCH_2CH_2]_wOCH_2CH_2-B$  with  $w$  between 0 and 10 and where B is OH, O-D or NH-D where D is a  $C_1-C_{12}$  alkyl group bearing an electrophilic function such as an isothiocyanate;
- $R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with  $x$  between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group; or  $R_{10}$  and  $R_{11}$  may represent an isoalkylidene group;

or



wherein  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_8$  and  $R_9$  are defined as above,

- $R_4$  is H or OH or  $OC_lH_{2l+1}$  with  $l$  between 2 and 6;
- $R_{10}$  is  $C_yH_{2y}$  with  $y$  between 1 and 12 or  $CH_2CH_2[OCH_2CH_2]_zOCH_2CH_2$  with  $z$  between 0 and 10; and

$R_{11}$  is H or OH or  $OC_xH_{2x+1}$  with  $x$  between 1 and 6 or Hal where Hal is Cl, Br, F or I; or an acetoxy group or a sulfonate ester group or an oxo group ;

and all stereoisomers and optical isomers thereof,

or their pharmaceutically acceptable salts, in the preparation of a pharmaceutical composition useful in a prophylactic or curative treatment of the malaria in the hepatic stage.

12. Pharmaceutical composition comprising a compound as defined in claim 10, or their pharmaceutically acceptable salts, together with at least one second anti-malarial compound a pharmaceutically acceptable vehicle.
13. Pharmaceutical composition comprising at least a compound as defined in claim 1, or their pharmaceutically acceptable salts and a pharmaceutically acceptable vehicle.
14. Pharmaceutical composition according to claim 12 or 13, wherein the compound of formula I is present in an amount of between 0,001 and 50% by weight of the composition.
15. Pharmaceutical composition according to claims 12 to 14, wherein the vehicle is aqueous.
16. Pharmaceutical composition comprising an isolated abstract of *Strychnopsis thouarsii*.



European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number  
EP 04 29 1055

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